5-2010

Reproductive Ecology of Astragalus filipes, a Great Basin Restoration Legume

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REPRODUCTIVE ECOLOGY OF *ASTRAGALUS FILIPES*, A GREAT BASIN RESTORATION LEGUME

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

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UTAH STATE UNIVERSITY
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2010

ABSTRACT

Reproductive Ecology of *Astragalus filipes,* a Great Basin Restoration Legume

by

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Utah State University, 2010

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Astragalus filipes Torrey ex. A. Gray (Fabaceae) is being studied and propagated for use in rangeland restoration projects throughout the Great Basin. Restoration forbs often require sufficient pollination services for seed production and persistence in restoration sites. Knowledge of a plant's breeding biology is important in providing pollination for maximal seed set.

Reproductive output from four manual pollination treatments (autogamy, geitonogamy, xenogamy, and distant xenogamy) was examined in a common garden. Pod set, seed set, and seed germination were quantified for each of the treatments. Seed set from four wild populations was compared to that of an openly visited common garden array. *A. filipes* was found to be self-compatible, but to benefit greatly from outcrossing. Less seed germinated from distantly outcrossed treatments than for any other treatment, indicating possible outbreeding depression. Common garden plants set less seed per pod than any wild population, possibly due to a depauperate pollinator guild in the common garden.

Bees were surveyed at wild *A. filipes* populations to identify common pollinators. Solitary and social bee species were observed visiting *A. filipes* to estimate aspects of their pollination efficacies, particularly foraging tempo and frequency of stigmatic contact. The nesting biologies of bees that visit *A. filipes* were considered as a component of bee manageability. Bees in the genus *Osmia* (Megachilidae) dominated this pollinator guild. *Bombus nevadensis* queens were the fastest foragers; honey bees and native solitary bees did not differ in foraging tempo. Megachilid bees consistently contacted the stigma during foraging, but honey bees exhibited sideworking behavior, contacting stigmas far less frequently than any other bee species observed. Two solitary bee species (*Osmia bruneri* and *Hoplitis hypocrita*) are recommended as prospective pollinators for management in association with Great Basin rehabilitation efforts.

(65 pages)

ACKNOWLEDGMENTS

I have many people to thank for making this thesis possible. I would like to thank my research advisor, Dr. James Cane, for his guidance and support, and for providing funding that made this research possible. I would also like to thank my academic advisor, Dr. Ted Evans, and my committee member, Dr. Geno Schupp, for their accessibility and support all this time.

Fellow bee-labbers at the USDA-ARS Bee Biology and Systematics Lab provided a great deal of help during this process. Thanks to Melissa Weber, Joyce Knoblett, Stephanie Miller, Katie Swoboda, H. Ikerd, and Glen Trostle for their support in various aspects of this project. Terry Griswold helped with bee identification.

I also had help from some folks at the USDA-ARS Forage & Range Research Lab. Thanks to Doug Johnson and Kevin Connors for providing seedlings for our common garden, and for access to *A. filipes* experimental plots. Kishor Bhattarai and Shaun Bushman shared their *A. filipes* knowledge with me. Thanks also to Scott Jensen and Ann DeBolt with the Forest Service for supplying me with fruit from wild *A. filipes* populations.

My wonderful makeshift family here in Utah really made this thesis possible with all their help and love. Special thanks to the Stranges, Wilsons, Scovilles, Duffys, Ellen Klinger, Craig Faulhaber, Jake Davidson, and my fellow dancers for keeping me sane and providing endless support in all its forms.

Most importantly, I want to thank my family. Thanks to my parents for instilling in me a strong thirst for knowledge, an unending curiosity. Mom, this is where catching ladybugs for your insect collection has brought me. Thank you. Cora and Tyler, thank

you for making me laugh and reminding me what it is to play and for helping me to see the world with fresh eyes. Most importantly, a huge thanks to Josh Der for so very much: field help, proofreading, endless biology talk, friendship, love, and constant support. This thesis is possible all because of you.

This research was funded by Great Basin Native Plant Selection and Increase Project through the USDI-BLM Great Basin Restoration Initiative and the USDA-FS Rocky Mountain Research Station.

Kristal Marie Watrous

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CHAPTER 1

INTRODUCTION

The health and integrity of an ecosystem are often reflected in the relative biotic diversity found therein. Plant diversity is important for maintaining ecosystem functions such as nutrient cycling and community productivity (Davies and Svejcar 2008). Native forb diversity within a plant community should affect the diversity of the local bee community, which reciprocally affects the pollination services available to those plants. Additionally, healthy native plant populations in sagebrush steppe are more resistant to invasion than their depleted counterparts (Anderson and Inouye 2001), a factor worth considering in an area threatened by invasion of cheatgrass (*Bromus tectorum* L.) and other nonnative plants.

Current restoration efforts in the Great Basin were preceded and prompted by a recent history of human disturbance. Livestock were introduced to the Great Basin in the late 1800s; their hooves and grazing disturbed the herbaceous understory in much of the rangeland (Knapp 1996; Pellant et al. 2004). Eurasian cheatgrass invaded and has filled the understory niche in this disturbed landscape. Estimates of cheatgrass-dominated rangeland in the Great Basin now run as high as 10.1 million hectares (Pellant et al. 2004). Invasion of cheatgrass has increased fire frequency in the Great Basin from a 60-110 year interval to as frequently as every 3-5 years (Whisenant 1990). This altered fire regime is lethal to native shrubs such as sagebrush (*Artemisia tridentata* Nutt.) while providing favorable conditions for cheatgrass to form dense stands, greatly altering the local ecosystem (Ott et al. 2001). Dense cheatgrass stands significantly reduce the

diversity of native herbs in an area by outcompeting germinating seedlings through limiting water available near the soil surface (D'Antonio and Vitousek 1992).

In the 1950s, efforts were mounted to restore or rehabilitate the cheatgrass-invaded Great Basin rangelands. Restoration and rehabilitation are similar efforts with different goals and approaches; rehabilitation involves seeding or planting native or introduced species to rectify existing disturbances (e.g. post-fire), while restoration implies that native species will be reestablished in an area, and that introduced species will not be used (Monsen and McArthur 1995). Most work in the Great Basin has been toward rehabilitation of an area, sometimes with the eventual goal of restoration of the native plant community.

The goal in addressing post-fire disturbances is typically rehabilitation of the area to stabilize the soil, preventing runoff and wind erosion and protecting the watershed. Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) has been used for rapid soil stabilization as it establishes easily after a disturbance, competes with other nonnative grasses such as cheatgrass, and provides some livestock forage (Richards et al. 1998). Restoration of native plant communities is reportedly easier in areas planted with crested wheatgrass than in dense stands of cheatgrass (Cox and Anderson 2004), although recent extensive field trials are failing to displace established crested wheatgrass by cultivation or herbicides (Fansler and Mangold 2007, Hulet 2009). If a method for crested wheatgrass displacement can be determined, this plant may function as a good intermediate planting against cheatgrass invasion when eventual restoration of native plant communities is the objective. While nonnative grasses can be useful where rapid establishment is needed, restoration of the native plant community is desirable as "native

species are critical to maintaining and restoring the genetic and ecological integrity of western native ecosystems" (Richards et al. 1998).

The Great Basin Restoration Initiative (GBRI) was created by the U.S. Department of the Interior's Bureau of Land Management (BLM) to specifically address protection and restoration of native plant communities following extreme fire seasons in the 1990s (Monsen and Shaw 2001; Shaw et al. 2005). Reseeding mixes used by the BLM in the past few decades have included a combination of exotic grasses, native grasses and native shrubs, but with only a meager 0.5% of the mix comprised by native forb seed (Cane 2008). Ideally, seed mixes for restoration efforts should contain a mix of grass, shrub, forb and, where appropriate, tree seeds representing prevalent native species that existed in the area prior to disturbance (Stevens 2004). Native seed is desirable for restoration of Western rangelands, as native plants provide both habitat and forage that benefit wild animals including species of management concern (e.g. Sage Grouse, Sharp-tailed grouse, Blue grouse; Walker and Shaw 2005). Until recently, few native forbs have been cultivated for seed production. While shrub seeds are often collected from wild populations, wild harvest of native forb seed is impractical and unreliable, yielding small quantities of expensive seed (Cane 2008); cultivation is thus desirable for seed production of native wildflowers. The GBRI developed the Great Basin Native Plant Selection and Increase Project (GBNPSIP) as a collaborative effort to increase the supply of native plant seeds available for restoration (Shaw et al. 2005). Through the GBNPSIP, researchers established a list of desired native Great Basin plants that seemed practical for seed cultivation and ultimate use in restoration efforts.

One wildflower of particular interest for Great Basin restoration is *Astragalus filipes* Torrey ex. A. Gray (Fabaceae) (basalt milkvetch or threadstalk milkvetch). This native perennial legume is distributed throughout the Great Basin north to the Columbia Plateau, with disjunct populations in southern British Columbia, the San Bernardino mountains of Southern California and northern Baja California (Isely 1998). This *Astragalus* species has some key characters which make it ideal for re-seeding rangelands and for cultivated seed production. The nitrotoxins, swainsonine and selenium that render other *Astragalus* species toxic are very dilute or absent from *A. filipes*, making it a safe forage for livestock (Williams and Barneby 1977; Bhattarai et al. 2008). The extensive range of this species makes it a good candidate for use throughout the Great Basin, as locally-sourced seed accessions will likely be well adapted to area-wide conditions (Bushman et al. *in review*). Finally, the fruits of *A. filipes* are borne on erect racemes, makes them easily accessible for mechanical seed harvest by combine. The first tested germplasm of *A. filipes* (NBR-1) was recently released for commercial cultivation (Johnson et al. 2008), and a few native plant growers already have stands of *A. filipes* in cultivation (J. Cane *personal communication*). As *A. filipes* comes into greater commercial production, it is useful to know the breeding biology and pollination needs of this species in order to best manage both the plant and its pollinators for consistent and copious seed production.

Little is known for most *Astragalus* breeding biologies. A literature search reveals breeding biology records for 29 *Astragalus* species globally, accounting for <1% of the nearly 3270 species of *Astragalus* found worldwide (Frodin 2004). Known *Astragalus* breeding biologies span the range of self-fertility from self-compatible to self-

incompatible, benefitting from outcrossing to obligate outcrosser. One annual species (*A. cymbicarpos* Brot.) is even cleistogamous (Gallardo et al. 1993). The large diversity of breeding biologies within this genus, coupled with a lack of knowledge about most species, makes predicting the breeding biology of *A. filipes* unreliable at best.

Most papilionaceous legumes are bee pollinated (Faegri and van der Pijl 1979); it is therefore reasonable to predict that *A. filipes* will benefit from bee visitation. Growing a crop in cultivation for seed production often means providing a managed effective pollinator in sufficient numbers to ensure adequate pollination service. Honey bees (*Apis mellifera* L.) are the most commonly used agricultural pollinator in the United States, but Colony Collapse Disorder and the *Varroa* mite continue to plague honey bee populations nationwide (Cox-Foster et al. 2007). Farmers have begun to look to native bees for affordable and sustainable local pollination services (Bohart 1972; Winfree et al. 2007). Few species of bees are currently managed for pollination, but with over 16,000 bee species worldwide (Michener 2000) it is likely that many more species could be amenable to management for pollination services (Bohart 1972).

The objectives of this study were three-fold: 1) to determine the breeding biology of *A. filipes* through manual pollination treatments, and compare seed set from these treatments to that found in wild populations; 2) to identify wild pollinators of *A. filipes*; and 3) to assess the manageability of these pollinators, then recommend a native bee species for development toward pollination management on *A. filipes*.

In chapter two I address the question of *A. filipes* breeding biology. First, a literature review of known breeding biologies for *Astragalus* species is presented. Then I determined the breeding biology of *A. filipes* using manual pollination treatments in a

common garden to simulate self-pollination, intra-population outcrossing and inter-population outcrossing. I then compared seed set results from our openly visited common garden plants to seed counts from wild *A. filipes* populations. I also conducted a seed germination study using seeds produced from our manual pollination treatments to further examine possible sources of reproductive attrition.

In chapter three I identified and evaluated wild pollinators of *A. filipes*. I surveyed bees at wild and cultivated stands of *A. filipes* to identify common floral visitors. I then observed the most common bee species for frequency of stigmatic contact and foraging tempo on *A. filipes* as qualitative and quantitative measures of pollination efficacy. I recommended bee species for management consideration based on these and additional criteria.

Knowledge of the breeding biology and pollinators of *A. filipes* will aid in managing this milkvetch for maximal seed production.

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CHAPTER 2

BREEDING BIOLOGY OF THE THREADSTALK MILKVETCH ASTRAGALUS FILIPES (FABACEAE), WITH A REVIEW OF THE GENUS[1]

ABSTRACT

Astragalus L. (Fabaceae) is an enormous and diverse plant genus with a cosmopolitan distribution, but remarkably few breeding biologies are known for its member species. Threadstalk (or basalt) milkvetch, *Astragalus filipes* Torrey ex. A. Gray, is common and widespread throughout the US Intermountain West, including the Great Basin. It is being studied and ultimately propagated for extensive rangeland restoration projects throughout the sagebrush-steppe. Understanding the breeding biology of *A. filipes* will be necessary for reliable and consistent commercial seed production with this species. We examined reproductive output from four manual pollination treatments (autogamy, geitonogamy, xenogamy and distant xenogamy) in a common garden. As measures of fitness, we counted fruit and seed set, then germinated viable seeds, to assess reproductive output. This species is weakly self-compatible; xenogamous pollen transfer results in nine times more seed per pollination. Pollen transfer between geographically distant seed accessions resulted in a decrease in seed germination, but no difference in fruit or seed set. Cross-pollination by bees will be necessary for copious seed production by this species.

[1] Coauthored by Kristal Watrous and James H Cane.

INTRODUCTION

Plants in the legume family (Fabaceae *sensu lato* Lindl.) are of global economic and ecological significance. The Fabaceae is second only to the grasses in economic importance, and is only smaller than the Orchidaceae and Asteraceae in numbers of species. Papilionoid legumes are particularly valuable as ground cover, forage and food crops (Allen & Allen 1981).

The genus *Astragalus* L. is the largest of the flowering plant genera (Frodin 2004). Comprising some 3270 species, *Astragalus* is most diverse in the Sino-Himalayan region, Russia, the Andes mountains of South America, and western North America (Allen & Allen 1981; Isely 1998). Nearly 400 species of *Astragalus* occur in North America, with 156 species occurring in the Intermountain West alone (Barneby 1964). Plants in this genus are economically significant as a source of gum tragacanth, as indicators of selenium and uranium, and as toxic locoweeds in rangelands (Allen & Allen 1981). The more widespread and common species can support diverse elements of the region's pollinating bee communities as well (e.g. Green & Bohart 1975; Clement *et al.* 2006)

Remarkably few *Astragalus* breeding biologies are known for such a large and prevalent genus. A literature search revealed known breeding biologies for only 29 *Astragalus* species worldwide, accounting for <1% of all *Astragalus* species (Table 2-1). These few species have breeding biologies that span the range of self-fertility, from self-compatible to self-incompatible, sometimes xenogamous to obligately so. One annual species (*A. cymbicarpos* Brot.) is even cleistogamous (Gallardo *et al.* 1993). This range of breeding biologies within the genus, coupled with a general paucity of knowledge

about most species, makes predicting the breeding biology of a given *Astragalus* species dubious at best.

Astragalus filipes Torrey ex. A. Gray (threadstalk milkvetch or basalt milkvetch) is widespread in western North American (Isely 1998). It is being evaluated and propagated for seed to use in rehabilitation projects throughout its range (Shaw *et al.* 2005). Wild seed production is erratic and susceptible to beetle predation, making it more costly and unpredictable than cultivated seed production (Youtie & Miller 1986; Cane 2008). The first tested germplasm for *A. filipes* (NBR-1) was recently released for commercial cultivation (Johnson *et al*. 2008). Knowing a plant's breeding biology can be critical for consistent and copious production of farmed seed. We expect species that are good colonizers, as *A. filipes* is after fires, to be self-compatible (Kalin Arroyo 1981; Bhattarai *et al*. 2008). Additionally, plants like *A. filipes* with considerable genetic diversity within populations (B.S. Bushman, personal communication) often prove to be outcrossers; thus we expected *A. filipes* to be at least moderately self-compatible although likely to benefit from outcrossing.

MATERIALS AND METHODS

Natural history of A. filipes

Astragalus filipes ranges from the southern Great Basin northward into the Columbia Plateau, with some disjunct populations in southern British Columbia, the San Bernardino mountains of southern California, and northern Baja California (Barneby 1964; Isely 1998). In some parts of its range *A. filipes* is "one of the truly common astragali... often occurring in colonies of great extent, sometimes in such quantity as to

color the sagebrush hillsides with a wash of creamy, spicily fragrant blossoms" (Barneby 1964). Due to its lack of toxins for livestock (Williams & Barneby 1977) and its extensive ecological and geographic range, *A. filipes* shows promise for restoration use (Shaw *et al.* 2005; Bhattarai *et al*. 2008). The fruits of *A. filipes* are presented on erect racemes easily accessible for mechanical seed harvest by combine. These factors make *A. filipes* a good candidate species for cultivated seed production.

Plant acquisition

Seeds were collected from wild *A. filipes* populations in fall 2003 by Douglas Johnson and Kevin Connors (USDA-ARS-FRRL). Seed locations used for this experiment represent eight different Omernik Level IV Ecoregions (Omernik 1987; Table 2-2). Seeds were germinated, then transplanted into conetainers in January 2004. A pair of small common garden arrays was established at the USDA-ARS Bee Biology and Systematics Laboratory (BBSL), Logan, UT, USA. Their silty clay-loam soil was amended with pea gravel for improved drainage. Seedlings were planted out in May 2004 into holes cut in weed barrier fabric to simplify weed control.

Pollination treatments

In May 2005, one array of *A. filipes* plants was covered with a walk-in net field cage (6 x 6 x 2 m) to exclude pollinators and facilitate manual pollinations with minimal handling of the plants. In the caged array, three pollination treatments were assigned to separate tagged racemes on each plant, replicated for 24 plants. All flowers on a raceme received the same pollination treatment. Racemes were chosen prior to bloom, using those with at least five buds. Number of flowers per raceme varied, and a range of four

to 23 flowers were treated per raceme (mean=12 flowers). The three pollination treatments were as follows: 1) geitonogamy, in which pollen was transferred from flowers of an unmarked raceme to a recipient flower on the same plant; 2) xenogamy, in which flowers of the treatment raceme received pollen from another donor plant from within the same seed accession location as the recipient plant; 3) distant xenogamy, in which flowers of the treatment raceme received pollen from donor plants belonging to a different Ecoregion than that of the recipient flowers (Table 2-2, Figure 2-1). This distantly outcrossed treatment allowed us to examine the possibility of outbreeding depression by transferring pollen between populations too distant for manual pollen transfer in the wild.

We also retained one raceme per caged plant as a negative control treatment of autogamy, in which flowers were counted and marked but otherwise unmanipulated. All pollen was transferred with dead honeybees' dorsal thoraces serving as disposable fine brushes which we rubbed against the anthers of the donor flower and then the stigma of a recipient flower. A different honeybee was used for each raceme to minimize unwanted pollen transfer. Each treated flower was marked on the banner petal with indelible ink. Flowers were counted and pollinated every other day until the racemes produced no new flowers. Plants of the neighboring array were left uncaged as a positive control to evaluate pod and seed set resulting from incidental visits by bees available at BBSL. These plants served as our open visitation treatment.

Seed production

Once the fruits (pods) were mature, but just prior to dehiscence, the racemes were collected and returned to the laboratory. Total number of fruits per raceme was recorded,

as well as each pod's content of plump seeds. Aborted ovules were tiny and disregarded. Plump seeds were allowed to dry and mature at 25˚C for 2 months before being placed in cold storage (4˚C).

Seed germination

Seeds were stored dry in envelopes at 4˚C for 6 months. After storage, all plump seeds were poked with an insect pin to perforate the seed coat for better water permeation. Seeds from the same raceme were then placed together in a single well of a tissue culture plate submerged in distilled water imbued with a fungicide to prevent mold. These tissue culture plates were then placed in a dark cold room (4˚C) and the seeds were monitored for germination. We recorded if the radicle was protruding from the seed coat, and transplanted the germinating seed in a conetainer with a native soil mix.

Seed counts from wild populations

Seeds were collected in 2005 from four different wild *A. filipes* populations: Pequop Summit, Elko County, NV (2); Big Gulch, Custer County, ID; and King Hill, Elmore County, ID. We counted the seed contents of 100 pods for each population to establish a baseline of wild seed set against which we compared our open visitation seed set at the Logan common garden.

Data analysis

We tested the null hypothesis that frequency of fruit set is independent of manual pollination treatment with an RxC Test of Independence using a G-Test with William's correction (Sokal & Rohlf 1981). Rows were manual pollination treatments, and columns were counts of pods or no pods produced. Individual flowers were used as

independent replicates. We excluded open visitation data from this analysis. If significant, then fruit sets were compared in a pairwise manner with G-Tests. Only flowers with fruit set were used for seed set analyses. We compared pollination treatments for proportions of pods containing seeds using CATMOD categorical analysis (SAS Institute 2004) using a linear model that the proportion of seeded pods to compare manual pollination treatments with our distant xenogamy treatment. We used distant xenogamy for comparison, as there was no significant difference in proportion of seeded pods between it and our xenogamy treatment. We used the Kruskal-Wallis test on ranked seed counts per pod, excluding empty pods, to compare seed counts per pod across pollination treatments (Proc NPAR1WAY; SAS Institute 2004). Seed germination percentages were analyzed with General Linear Model (GLM) ANOVA. Seed germination treatment means were compared using REGWQ *a posteriori* tests. Seed counts for wild populations and our open visitation treatment were analyzed using GLM ANOVA. These data were transformed by adding one to each value then applying a cube root transformation. Seed count means were compared using REGWQ *a posteriori* tests.

RESULTS

Cross-pollination significantly increased fruit and seed set over the two self-pollination treatments (autogamy and geitonogamy). Frequency of fruit set differed among pollination treatments ($G_{[3, 1191]} = 12.38$, $P<0.0005$, Figure 2-2). Flowers of autogamy racemes were less likely to set fruit than the other three pollination treatments combined ($G_{adj} = 107.78$, $P<0.0005$). The frequencies of seedless pods differed between pollination treatments ($G_{[4, 835]} = 255.49$, $P<0.0001$, Figure 2-3). Flowers of autogamy

racemes were less likely to produce pods with at least one seed than were geitonogamy racemes (G=5.13, P=0.0236). Geitonogamy racemes set more seedless pods than xenogamy racemes (G=11.20, P=0.0008). Open visitation racemes set fewer seedless pods than xenogamy racemes (G=48.19, P<0.0001). Ranked seed counts per pod also differed between pollination treatments ($H_{[3, 204]}$=10.69, P=0.0135, Figure 2-4).

Percent germinable seed per raceme varied with pollination treatment ($F_{[4,59]}$ = 3.18, P=0.0197, Table 2-3). Distant xenogamy seeds were significantly less likely to germinate than seeds from geitonogamy, xenogamy and open visitation treatments. Autogamous seeds were not evaluated as so few were produced.

Seed set was significantly different between the openly pollinated populations we compared ($F_{[4,658]}$ = 12.86, P<0.0001, Figure 2-5). Openly visited plants in our common garden at BBSL set significantly fewer seeds per pod than did *A. filipes* plants from four wild populations. Seed set did not differ significantly between the four wild populations.

DISCUSSION

Breeding biologies of papilionoid legumes are diverse, ranging from cleistogamous to obligately xenogamous (Kalin Arroyo 1981). Though moderately self-compatible, *A. filipes* and many other papilionoid legumes are bee-pollinated (Kalin Arroyo 1981), and benefit strongly from outcrossing facilitated by bee visitation. This combination of self-compatibility while favoring outcrossing is often classified as a "mixed mating system" (Neal & Anderson 2005).

The autogamous (unmanipulated) treatment yielded significantly less fruit and seed set than all other treatments, indicating that most *A. filipes* seed production does not

result from mere autopollination. Cross-pollination yielded nine times more seed than autogamy (93 vs 11 seeds per 100 flowers) and three times more than geitonogamy (32 seeds per 100 flowers). Hence, although a colonizing individual can produce some progeny, even in the absence of pollinators, a substantial fraction of seed from well-pollinated plants in a population should result from cross-pollination.

Openly visited flowers yielded the most seed per fruit, significantly more than the manual pollination treatments. These flowers likely received more frequent visitation and pollen deposition than did our manually pollinated flowers, each of which we pollinated only once. We observed bumble bee queens (*Bombus huntii* Greene, *B. fervidus* (Fabricius)) and workers (*B. huntii*) foraging at these uncaged plants frequently throughout the day. As with our results, Geer and Tepedino (1993) found that bees were superior pollinators to the experimenters for another *Astragalus* species. Despite the considerable fruit and seed set conferred by bumble bees in our common garden, our openly visited seed production was still less than that found in wild *A. filipes* populations (Figure 2-5). Perhaps our *Bombus* -dominated pollinator guild was less effective than the *Osmia*-dominated guilds we have found associated with wild *A. filipes* throughout its range. Additionally, *A. filipes* plants may perform better in their respective native locations than in our common garden; most wild plants surveyed were larger and likely older than our young transplants.

A smaller proportion of distant xenogamy seeds germinated compared with those from geitonogamy, xenogamy and open visitation treatments. This negative effect of pollen transfer distance on seed germination rates raises concern of possible outbreeding depression (Price & Waser 1979). When releasing germplasm for increase under

cultivation and eventual seeding back into the landscape, questions arise regarding the reproductive interaction of introduced genetic material (often from distant populations) on existing conspecific plant populations within a restoration area (Monsen & Shaw 2001; Hufford & Mazer 2003; McKay *et al.* 2005; Becker *et al.* 2006). However, experimental trends with small sample sizes such as ours may not reasonably represent conditions at a larger scale. According to B.S. Bushman (2008, personal communication) most detected genetic variability in *A. filipes* is within populations, as is expected for a primarily outcrossing species. Outbreeding depression is likely minor for species with genetically diverse populations.

Remarkably few *Astragalus* breeding biologies are known despite the enormity of this genus (Table 2-1). A literature search revealed information on breeding biologies for only 29 *Astragalus* species worldwide, accounting for <1% of total *Astragalus* species. Of those species with known breeding biologies, half (15) of these taxa are self-compatible. Of these self-compatible taxa, five are widespread and ten have restricted distributions. Four more species are self-compatible but benefit from xenogamous pollen transfer. One-third (10) of the taxa are obligate outcrossers: seven are geographically widespread and three are restricted. Most threatened or endangered astragali are self-compatible, though one endangered species in Arizona, U.S.A., *A. cremnophylax* Barneby, varies dramatically from inbreeding to self-incompatible between locations and varieties (Allphin *et al*. 2005). Widespread species seem to have no distinct pattern of breeding biology, with five self-compatible taxa, seven obligate outcrossers, and three taxa benefitting from outcrossing. One unusual annual species from Spain and North Africa, *A. cymbicarpos* Brot., is even cleistogamous in some populations (Gallardo *et al.*

1993). Predicting the breeding biology of a single *Astragalus* species is speculative owing to the diversity of breeding biologies and our lack of knowledge about most species within this genus.

As in other papilionoid legumes, bees commonly visit *Astragalus* flowers for nectar and pollen (Kalin Arroyo 1981). Among the ½ million bees pinned in the collections of the BBSL, 3400 specimens representing 192 bee species are labeled as having been taken at *Astragalus*. Among these floral hosts are 55 different species of *Astragalus*. No species of *Astragalus* is given for one-third of the pinned specimens, probably due to the daunting identification challenge. Perhaps for this reason, no bee in the BBSL collection was recorded from *A. filipes* until this study.

We have collected the fauna of bees that visit *A. filipes* around the Great Basin and Snake River Plains of the U.S. Intermountain West. We sampled bees visiting 24 populations of *A. filipes* growing in sagebrush basins and dry coniferous forests across five states (California, Idaho, Nevada, Oregon, Utah) during May and June. Few of the visiting individuals and species were social bees (bumblebees mainly) and none appears to be a specialist (=oligolege) on just *Astragalus*. Fully half of the 353 bees we sampled at *A. filipes* belong to 34 species of *Osmia* bees (Megachilidae). This remarkable diversity represents ¼ of the named species of *Osmia* in all of North America (Cane *et al.* 2007). Abundant and diverse *Osmia* have been observed for other *Astragalus* species at other locations, with *Osmia* comprising more than 60% of the total flower visitor fauna sampled from three other *Astragalus* species over three years (Geer *et al*. 1995). As in that study and others (Green & Bohart 1975; Clement *et al.* 2006), bees of the genera

Anthidium, Bombus, Eucera and *Hoplitis* were also regular members of the guild of bees sampled at *A. filipes* flowers.

We found that *A. filipes* has a mixed mating system, as it is self-compatible but benefits greatly from outcrossing. Bees of several genera were regularly observed visiting *A. filipes* at numerous locations. Bees apparently transfer pollen better than manual pollination by researchers. Wild populations of *A. filipes* set significantly more seed per pod than did our open visitation treatment. Our research adds to the meager body of literature on breeding biologies of *Astragalus*, the largest genus of flowering plants. With this knowledge, *A. filipes* plants can be grown for maximal seed production and viability.

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Table 2-1. Literature review of Astragalus breeding biologies. Letters in parentheses indicate a Threatened (T) or Endangered (E) status. Pollinator genera are included, if known.

Astragalus species	Breeding biology	Distribution	Pollinator genera	Reference
A. alpinus L.	obligate outcrosser	Widespread, circumpolar subarctic & arctic	*Bombus*	Kudo & Molau 1999
A. americanus (Hook.) M.E. Jones	obligate outcrosser	Widespread, W.N.America	*Bombus*	Kudo & Harder 2005
*A. ampullarioides (*S.L. Welsh) S.L. Welsh	self-compatible	Restricted (E), W.N.America	*Anthophora*, *Bombus*, *Dialictus*, *Osmia*	Tepedino 2005
A. australis (L.) Lam. var. *olympicus* Isely	moderately self-compatible	Restricted (T), W.N.America	*Anthidium*, *Bombus*, *Megachile*, *Osmia*	Kaye 1999
A. canadensis L.	obligate outcrosser	Widespread, W.N.America	*Bombus*	Platt *et al*. 1974
A. cibarius Sheldon	outcrossing beneficial	Widespread, W.N.America	*Anthophora*, *Bombus*, *Eucera*	Green & Bohart 1975
A. cicer L.	outcrossing beneficial	Widespread crop, cosmopolitan	*Apis*, *Bombus*, *Megachile*	Richards 1986
A. cremnophylax var. *cremnophylax,* North Rim population	self-compatible	Restricted (E), W.N.America	unknown	Allphin *et al*. 2005
A. cremnophylax var. *cremnophylax,* South Rim population	obligate outcrosser	Restricted (E), W.N.America	unknown	Allphin *et al*. 2005
A. cremnophylax var. *herronii*	self-compatible	Restricted (E), W.N.America	unknown	Allphin *et al*. 2005
A. cremnophylax var. *myriorraphis*	outcrossing beneficial	Restricted (E), W.N.America	unknown	Allphin *et al*. 2005
A. cymbicarpos Brot.	cleistogamous and chasmogamous are self-compatible	Restricted, Iberian peninsula & North Africa	unknown	Gallardo *et al*. 1993, 1994
A. edulis Durieu ex Bunge	self-compatible	Widespread, Europe to W. Asia	unknown	Gallardo *et al*. 1994
A. epiglottis L. subsp. *epiglottis*	self-compatible	Widespread, Africa, Europe & Middle East	unknown	Gallardo *et al*. 1994

Astragalus species	Breeding biology	Distribution	Pollinator genera	Reference
A. epiglottis L. subsp. *asperulus* (Dufour) Nyman	self-compatible	Restricted, Spain	unknown	Gallardo *et al*. 1994
A. hamosus L.	self-compatible	Widespread, Europe	unknown	Gallardo *et al*. 1994
A. holmgreniorum Barneby	self-compatible	Restricted, W.N.America	*Anthophora*, *Apis*	Tepedino 2005
A. humillimus A. Gray	self-compatible	Restricted (E), W.N.America	*Apis*, *Eucera*, *Osmia*	Geer *et al*. unpublished
A. kentrophyta var. *tegetarius* (S. Wats.) Dorn	obligate outcrosser	Widespread, W.N.America	*Anthidium*, *Osmia*	Geer & Tepedino 1993; Geer *et al*. 1995
A. linifolius Osterh.	self-compatible, moderately autogamous	Restricted, W.N.America	*Anthophora*, *Apis*, *Bombus*, *Osmia*	Karron 1987, 1989
A. lonchocarpus Torrey	self-compatible	Widespread, W.N.America	*Anthophora*, *Apis*, *Bombus*, *Osmia*	Karron 1987, 1989
A. miser var. *oblongifolius* (Rydb.) Cron.	obligate outcrosser	Widespread, W.N.America	*Bombus*, *Osmia*	Geer & Tepedino 1993; Geer *et al*. 1995
A. monoensis Barneby	obligate outcrosser	Restricted, W.N.America	*Anthidium, Apis, Bombus, Hoplitis, Osmia*	Sugden 1985
A. montii Welsh	self-compatible	Restricted (E), W.N.America	*Anthidium*, *Osmia*	Geer & Tepedino 1993; Geer *et al*. 1995
A. osterhouti M.E. Jones	moderately self-compatible	Restricted, W.N.America	*Anthophora*, *Apis*, *Bombus*, *Osmia*	Karron 1987, 1989
A. pectinatus (Hook.) G.Don	obligate outcrosser	Widespread, W.N.America	*Anthophora*, *Bombus*, *Osmia*	Karron 1989
A. striatus Nutt.	obligate outcrosser	Widespread, W.N.America	*Bombus*	Kudo & Harder 2005
A. tennesseensis Chapman	obligate outcrosser	Restricted, Midwest US	*Apis*, *Bombus*, *Xylocopa*	Baskin *et al*. 1972
A. utahensis (Torr.) Torr. & A.Gray	outcrossing beneficial	Widespread, W.N.America	*Anthophora*, *Bombus*, *Eucera*	Green & Bohart 1975

Table 2-2. Plant accession locations for manual pollination treatments. Distant xenogamy treatments were applied across different level IV Ecoregions (Omernik & Gallant 1986, McGrath *et al*. 2002, Bryce *et al*. 2003, Thorson *et al*. 2003). Superscript letters indicate seed accessions which were crossed in distant xenogamy treatments, and correspond to location numbers in Figure 1.

Site name	County, State	Ecoregion IV	Ecoregion III
Ellensburg[1]	Kittitas, WA	10g - Yakima Folds	Columbia Plateau
Clarno[1]	Wasco, OR	11a - John Day/Clarno Uplands	Blue Mountains
Otley Ranch[2]	Harney, OR	80g - High Lava Uplands	Northern Basin & Range
Mountain City[2]	Elko, NV	80a - Dissected High Lava Plateau	Northern Basin & Range
Warrior Mine[3]	Nye, NV	13v - Tonopah Sagebrush Foothills	Central Basin & Range
Big Gulch[3]	Custer, ID	17e - Barren Mountains	Middle Rockies
Black Mountain[4]	Owyhee, ID	80f - Owyhee Uplands & Canyons	Northern Basin & Range
Champs Flat[4]	Lassen, CA	342Bd - Cottonwood – Skedaddle Mountains	Northern Basin & Range

Table 2-3. Mean proportion (± standard error) seed germination per raceme for four pollination treatments. Treatments were conducted at BBSL in 2005. N = number of plants. Letters following treatment means indicate statistical difference ($P \leq 0.05$).

Pollination treatment	N	$X \pm s_x$	Range
Open Visitation	22	$0.73^a \pm 0.33$	0 — 1.0
Geitonogamy	11	$0.72^a \pm 0.38$	0 — 1.0
Xenogamy	16	$0.80^a \pm 0.24$	0.25 — 1.0
Distant Xenogamy	15	$0.43^b \pm 0.29$	0 — 1.0

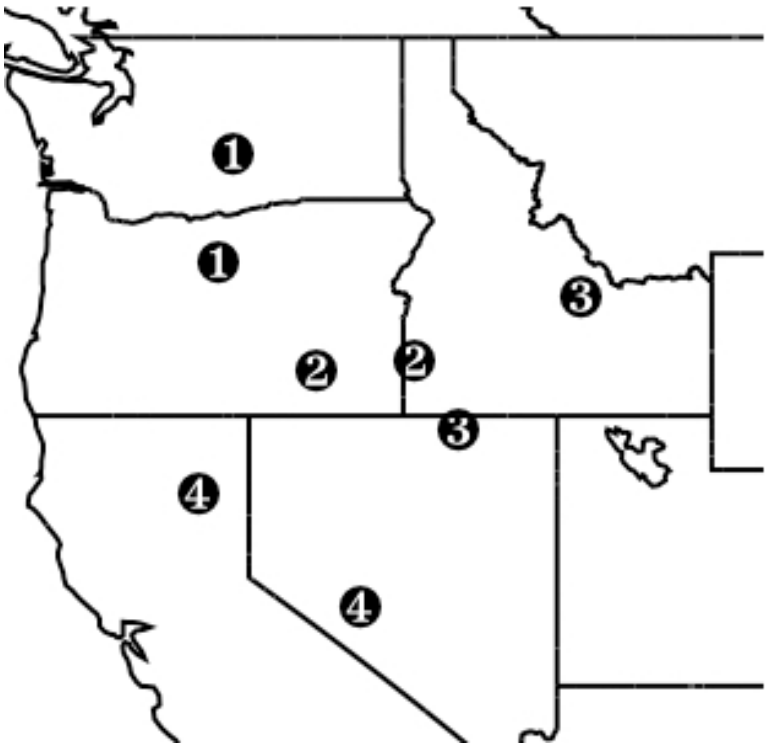

Figure 2-1. Seed source locations for common garden plants. Pairs of numbers indicate populations that were crossed in our distant outbreeding treatment. Populations crossed are from different Omernik level IV Ecoregions.

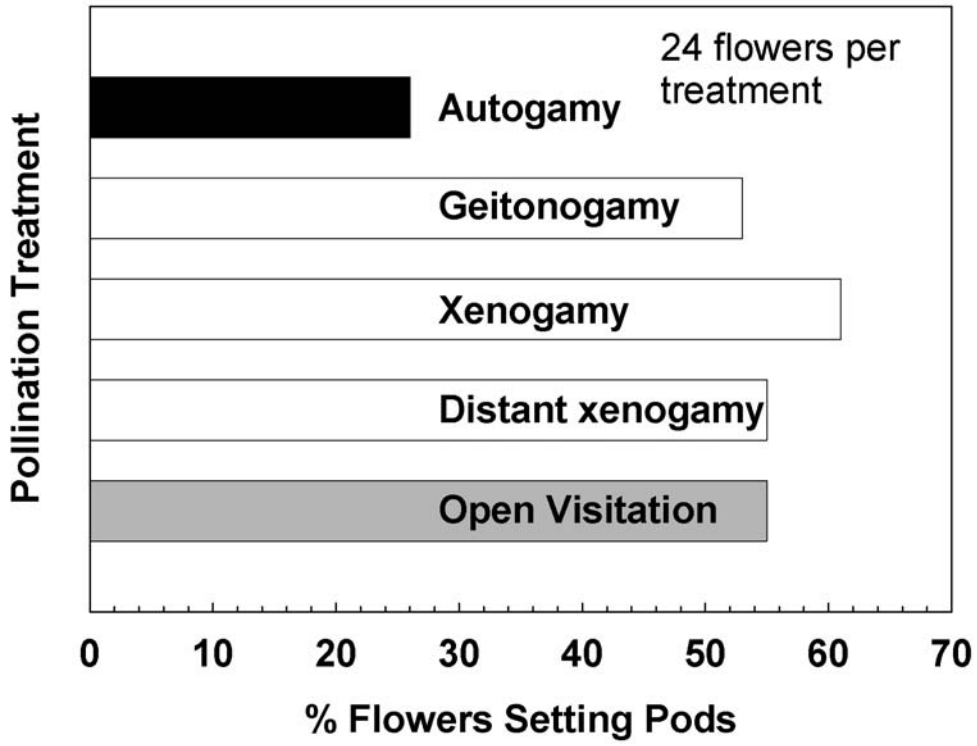

Figure 2-2. Proportion pod set per flower pollinated. Different fills denote a significant difference between pollination treatments.

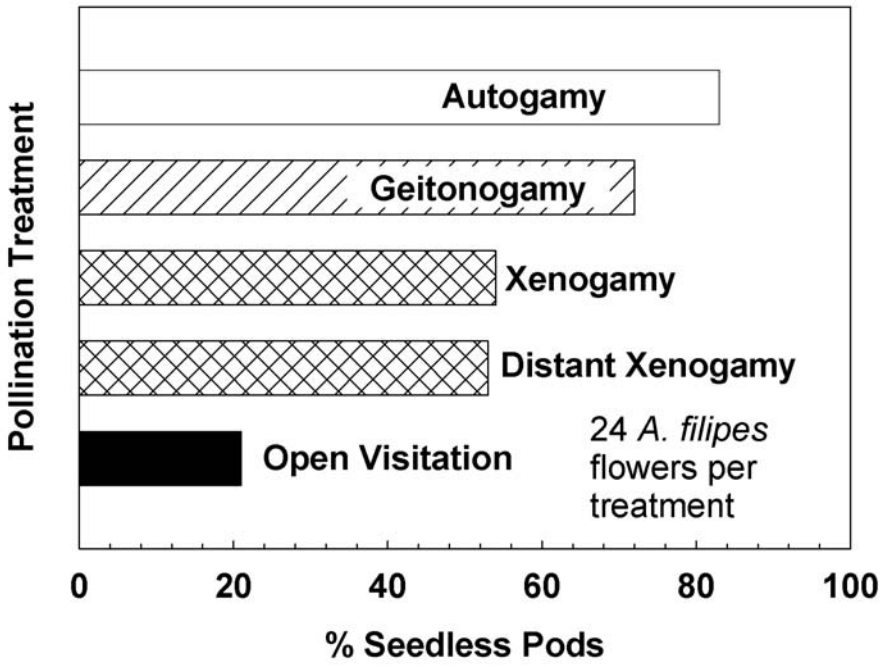

Figure 2-3. Proportion seedless pods per pod formed. Different fills denote a significant difference between pollination treatments.

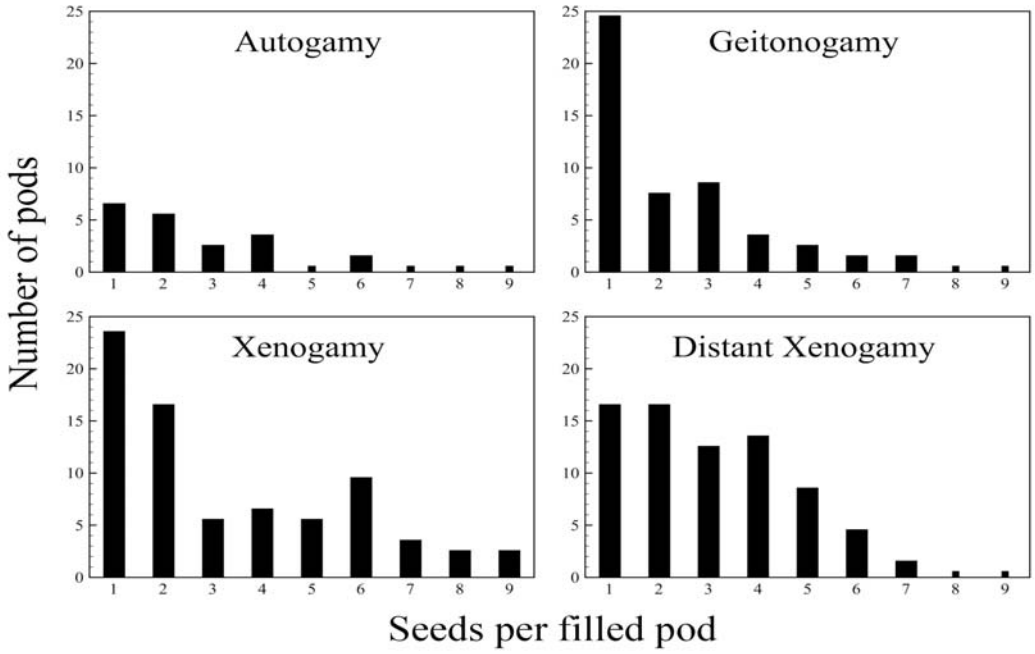

Figure 2-4. Ranked seeds per pod, compared across manual pollination treatments.

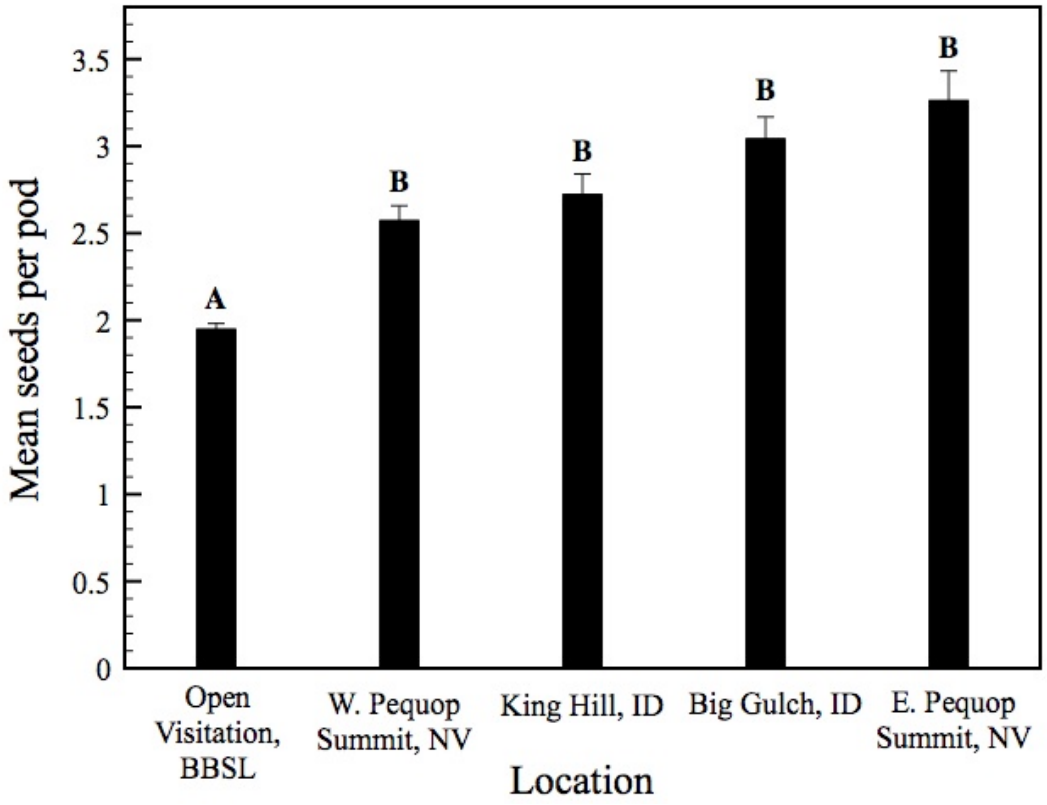

Figure 2-5. Mean count of seeds per seeded pods from wild populations and our open visitation plants. Data used are untransformed. Error bars show standard error.

CHAPTER THREE

AN ASSESSMENT OF THE NATIVE BEE FAUNA AND POLLINATOR EFFICACY ON ASTRAGALUS FILIPES, A GREAT BASIN RESTORATION SPECIES.

ABSTRACT

When considering plants for Great Basin rangeland rehabilitation it is important to ensure that sufficient pollination services will be available for seed production and continued survival of these wildflowers. Threadstalk milkvetch, *Astragalus filipes* Torrey ex. A. Gray (Fabaceae), is one such wildflower under consideration for restoration use. Little is known about the pollinators of this plant. Wild bees were surveyed at flowers of 24 *A. filipes* populations to identify common pollinators. Common bee species were observed on cultivated *A. filipes* to measure aspects of pollinator efficacy by quantifying foraging tempo and frequency of stigmatic contact. Bees in the genus *Osmia* dominated this pollinator guild. *Bombus nevadensis* queens were the fastest foragers. Honey bees exhibited sideworking behavior, contacting stigmas far less frequently than any other bees observed. I recommend *Osmia bruneri* and *Hoplitis hypocrita* as promising pollinators for management in association with Great Basin rehabilitation efforts. Native bees can provide reliable local pollination services, and may be a good alternative to managed honey bee or bumble bee colonies for pollination of native plant species.

INTRODUCTION

There has been a recent increased effort to rehabilitate burned Great Basin rangelands with native plants. As more native wildflower seed is added to the reseeding mixes, it becomes important to ensure that sufficient pollination services will be available for seed production and continued survival of these wildflowers. Many of these plants rely on bee visitation for seed set (Cane 2008), yet little is known of bee communities within the Great Basin (but see Bohart and Knowlton 1973, Rust et al. 1983, Frohlich et al. 1988, Wilson et al. 2008), making it difficult to predict how these pollinators will respond to fire and post-fire reseedings.

One particular native forb that is poised for rehabilitation use is *Astragalus filipes* Torrey ex. A. Gray (threadstalk milkvetch, Fabaceae). While I have determined that *A. filipes* requires a pollinator and benefits from outcrossing (Chapter 2), I do not know the identity of its wild pollinators. Pollination studies conducted on other *Astragalus* species show a wide array of dominant bee genera. Green and Bohart (1975) found that large, long-tongued bees (*Anthophora*, *Bombus* and *Eucera*) were the primary pollinators of *Astragalus cibarius* and *A. utahensis.* These bee genera are found on some other widespread Great Basin *Astragalus* species, but at much lower numbers than the ubiquitous *Osmia* (Rust et al. 1983, Geer and Tepedino 1993, Geer et al. *unpublished data*). A highly diverse genus of solitary bees, *Osmia* species seem to comprise an important component of various *Astragalus* pollinator guilds in western North America, including those of non-native milkvetches cultivated in the Columbia Basin (Clement et al. 2006).

Identifying the common floral visitors of *A. filipes* will give me baseline information on one Great Basin bee guild likely to be affected both by fire and subsequent rehabilitation efforts. Because the natural history of bees is highly variable, sometimes even within a single genus (Cane et al. 2007), a survey of floral visitors will allow me to assess how these bees may be susceptible or resilient to disturbance such as fire. Additionally, information on dominant pollinator identity may help optimize placement of expensive seed used in rehabilitation (e.g. placed closest to burn margins, or clumped if mostly pollinated by specialists).

In this study I surveyed floral visitors of wild *A. filipes* populations. I then assessed a subset of these bees for their flower handling abilities to determine their pollination promise.

METHODS

Wild floral visitors

Bees were sampled on wild and cultivated *A. filipes* populations in five western states in 2003, 2004, and 2006. Plant populations in Oregon, Idaho, California, Nevada, and Utah were surveyed (Figure 3-1). Plants were surveyed between 1000h and 1630h, when bees are most active during the day. Bees were netted as observed, and species unidentifiable in the field were retained as voucher specimens, identified, and deposited in the U. S. National Pollinating Insects Collection at the USDA-ARS Bee Biology and Systematics Laboratory in Logan, UT. Plant vouchers were collected and deposited at the Intermountain Herbarium at Utah State University (UTC).

Flower handling

For one quantitative component of pollinator efficacy, I observed the foraging tempo of bees visiting *A. filipes* in cultivation at three sites in Cache County, Utah, these being: 1) USDA field plots in Millville, 2) Evans experimental farm, and 3) a common garden at the USDA-ARS Bee Biology and Systematics Laboratory in Logan, Utah (for common garden setup see Chapter 2). Individual freely-foraging wild female bees were timed from their first contact with the first flower until they contacted the sixth flower visited. This was considered as five complete flower visits and transits. Observations were conducted between 1000h and 1630h on sunny days over 65° F with wind less than ten miles per hour. Observations were recorded for as many individuals and species as possible, but only species with over 50 observed visits were included in data analyses. Bees unidentifiable in the field were vouchered, identified, and deposited in the U. S. National Pollinating Insects Collection.

As a qualitative component of pollinator efficacy, bees were observed for frequency of stigmatic contact while visiting flowers of *A. filipes* in cultivation at Millville and Evans Experimental Farm, Cache County, Utah. I tallied the numbers of flowers a single bee visited and the proportion of those flowers in which stigma contact could be observed. Observations with unclear stigma contact were discarded. A minimum of four flower visits was required for a bee to be included in the data. Observations were conducted between 1000h and 1630h on sunny days over 65° F with wind less than ten miles per hour. Bees unidentifiable in the field were vouchered and deposited in the U. S. National Pollinating Insects Collection.

Data analysis

Foraging tempo data were log-transformed to meet assumptions of normality and analyzed with a General Linear Model ANOVA. I tested the null hypothesis that mean foraging time is independent of pollinator species. Where the overall ANOVA was significant, mean foraging times per species were compared using Ryan-Einot-Gabriel-Welsch-Q (REGWQ) *a posteriori* tests (SAS 2004). Stigmatic contact frequency data were sorted into four categories before analysis: for class 1, stigmatic contact was observed in less than 70% of visits; 70-89% of visits in class 2; 90-99% of visits in class 3; 100% stigmatic contact observed in class 4 visits. I tested the null hypothesis that frequency of stigmatic contact is independent of pollinator species with an RxC Test of Independence using G-Test with William's correction (Sokal and Rohlf 1981), with bee species as rows and stigmatic contact classes as columns.

RESULTS

Wild floral visitors

At *A. filipes* flowers 77 species of bees were collected (Table 3-1). Bees in the genus *Osmia* comprised 34 of the 77 species collected (Figure 3-2). Most species were represented in our surveys by five or fewer individuals (71%), with singletons comprising 30% of our species collected. Additionally, most bee species listed (68%) were only collected at one or two locations out of 24 locations surveyed (Figure 3-3). Listed by number of localities, six species were most widely collected: *Osmia bruneri* Cockerell (13 locations/32 individuals), *Hoplitis hypocrita* (Cockerell) (9/21), *Eucera frater* (Cresson) (9/27), *Bombus huntii* Greene (8/18) *Osmia nigrifrons* Cresson (7/15) and

Bombus centralis Cresson (7/12). Counties where these bees were collected are shown in Table 3-3.

Flower handling

Comparing foraging tempos for those bee species with over 50 observed visits, *Bombus nevadensis* Cresson queens were significantly faster than all other species (*Apis mellifera* L., *Hoplitis hypocrita, Osmia bruneri, O. sanrafaelae* Parker) ($F_{[4,195]} = 20.18$, $P<0.0001$; Table 3-2). When foraging tempos were compared for bee species with over 100 observed visits (all above species, with *B. nevadensis* excluded) there was no significant difference in foraging tempos between the four remaining species ($F_{[3,184]} = 1.86$, $P=0.138$).

I compared the frequency of stigmatic contact at *A. filipes* flowers across five bee species. The species with over 60 observed visits each included *Apis mellifera, Bombus huntii*, *B. nevadensis* queens, *Hoplitis hypocrita* and *Osmia bruneri*. Stigmatic contact frequency differed significantly between these five bee species ($X^2_{[4, 0.05]}=14.86$, $P<<0.05$, Figure 3-4). Honey bees contacted stigmas significantly less often than did the four other bee species ($X^2_{[1, 0.05]} = 7.879$, $P<<0.05$, Figure 3-5). I then removed honey bees from the analysis and compared the frequency of stigmatic contact of the remaining four species. These species (*B. nevadensis*, *B. huntii*, *H. hypocrita* and *O. bruneri*) showed no significant difference in frequency of stigmatic contact ($X^2_{[3, 0.05]} = 12.838$, $P>0.05$).

DISCUSSION

As with the few other Great Basin *Astragalus* species studied, *Osmia* were the dominant floral visitors collected on *A. filipes*. Over half of the species collected belong to this single diverse genus, representing ¼ of the named species of *Osmia* in all of North America (Cane et al. 2007). Abundant and diverse, *Osmia* have been observed for other *Astragalus* species, comprising more than 60% of the total flower visitor fauna sampled from three other western *Astragalus* species over three years (Geer et al. 1995). Most of the bee species collected at *A. filipes* were neither abundant nor widely distributed, a common occurrence in bee surveys (Williams et al. 2001).

I assessed the most common *A. filipes* visitors for their floral handling abilities. Since not all floral visitors are pollinators, I used these measures as a first proxy for beginning to evaluate pollination ability. I first measured the foraging tempo of dominant bee species on *A. filipes*. Bees with a faster foraging tempo will visit more flowers in a set amount of time.

I found that *Bombus nevadensis* queens were the fastest foragers of the bees studied. These results match those of Richards (1996) studying another *Astragalus*, who found that *B. nevadensis* were significantly faster foragers than honey bees on *Astragalus cicer* L.. Though a fast and effective foraging species, *B. nevadensis* is not currently manageable (J. Strange *personal communication*). Honey bees forage faster on *A. filipes* than on *A. cicer* (6.0 sec/fl on *A. filipes*, 8.6 - 17.3 sec/fl on *A. cicer*). Richards (1996) found that honey bees were the slowest visitors on *A. cicer*, alfalfa (*Medicago sativa* L.), sainfoin (*Onobrychis viciaefolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.), all papilionoid legumes. In contrast, I found no significant difference in the foraging tempos

of honey bees and native solitary bees on *A. filipes.* Foraging tempos for *Hoplitis hypocrita* and both *Osmia* species were close to those recorded for another solitary megachilid species, the alfalfa leaf-cutter bee (*Megachile rotundata*) on *A. cicer* (Richards 1996).

Stigmatic contact is required for proper pollen transfer and is another component of pollinator efficacy. Megachilid bees visiting papilionoid flowers often depress the keel, exposing the staminal column and stigma. These bees then tamp their abdomen on the sexual column, contacting the anthers and stigma with their abdominal scopae (Rittenhouse and Rosentreter 1994, Swoboda 2007). This behavior resulted in the high stigmatic contact frequency we observed in *O. bruneri* and *H. hypocrita*.

I observed honey bees "sideworking" *A. filipes* flowers (Bohart 1957), completely avoiding depressing the keel and thus likely missing the stigma altogether. Honey bees made significantly less frequent stigmatic contact than did the other species observed. This sideworking behavior seems to be a fairly common observation of honey bee behavior on legume flowers (Webb and Shand 1985, Heenan 1998, Etcheverry et al. 2003, Camacho et al. 2004). Westerkamp (1991) proposed that honey bees may not be effective legume pollinators in general due their generalist nature. Of the common floral visitors I assessed for pollination ability, it seems that most are effective enough floral handlers to be considered pollinators of *A. filipes.*

Flowers of *A. filipes* require pollen transfer by bees for optimal seed production (Chapter 2). Ensuring sufficient pollination of newly established plants in rehabilitated areas will be key for continued plant survival and reproduction. Thus knowing the likelihood of native pollinator survival or recolonization post-fire, as well as the potential

for supplementing pollination services, can foreshadow reproductive success of seeded populations.

A bee species' nesting biology will greatly affect its ability to survive a fire. Twig or woody cavity-nesting bees (some *Osmia, Hoplitis*) and perhaps some ground nesting bees with shallow burrows (some *Megachile, Trachusa*) will fall victim to direct combustion or heating from fire (Potts et al. 2003). Ground nesters with fairly deep burrows (beyond 10-20cm deep; *Bombus, Eucera*, some *Osmia*) will probably escape the heating effects of fire and survive (Potts et al. 2003). In fact, nests as shallow as 5cm may be deep enough to escape the effects of all but the most intense fires (J. Cane *personal communication*). The species collected on *A. filipes* represent a range of nesting biologies from surface (2%) and cavity (28%) to ground nesting (41%). Nearly one-third of the species collected (28%) have an unknown nesting biology, making it difficult to predict their fate in a fire.

A burned area may suffer reduced pollinator availability immediately post-fire due to loss of food sources and nesting substrate. However, given the range of nesting possibilities and the ability of bees to fly considerable distances (Gathmann and Tscharntke 2002), it seems reasonable to expect that a burned or rehabilitated area will suffer minimally from pollinator loss in the year(s) following a fire, at least in the small fires (<500 hectares) studied by Potts et al. (2003).

It may be possible to reintroduce some native bees to a burned area if supplemental pollination is necessary. I recommend use of native cavity-nesting bees, as only these can be trap-nested for relocation. The ideal bees for pollination supplementation would be native, easily managed, and effective at pollinating the target

plant or group of plants. A generalist pollinator will be more likely to visit a variety of flowers, thus benefiting more plants in a rehabilitated area.

From our survey of *A. filipes* visitors I recommend *Osmia bruneri* and *Hoplitis hypocrita* as prospective pollinators for management in association with Great Basin rehabilitation efforts. These two species have been collected in trap-nests (Clement and Rust 1976, Frohlich 1983), and are known cavity nesters. Both species were collected extensively throughout the range of *A. filipes*, at thirteen and nine locations, respectively. These bees behave similarly on *A. filipes*, exhibiting similar foraging tempos and a high frequency of stigmatic contacts. The basic nesting biology of *O. bruneri* is already known, and there is ongoing research at the Bee Biology and Systematics Lab for management development of this candidate species (Frohlich 1983, Frohlich and Tepedino 1986). *Osmia bruneri* shows great promise as a managed pollinator of native wildflowers, *A. filipes* included.

Less is known about the management potential of *Hoplitis hypocrita*. There have been no known attempts to release *H. hypocrita* and carefully monitor re-nesting in an area. Concern about re-nesting in a desired location is warranted, as other *Hoplitis* species have been observed to abscond from nest release sites, failing to remain in the desired location (personal observation). Failure to re-nest in the release area is undesirable for a prospective managed bee species. More research is needed to determine the likelihood of *H. hypocrita* re-nesting at a release site.

Continued research is recommended to develop *O. bruneri* and *H. hypocrita* for pollination management on Great Basin wildflowers. This list of known wild pollinators of *A. filipes*, along with additional research in floral preference, nesting needs, foraging

tempo and frequency of stigmatic contact provide additional information for possible management of other native bee species in a rehabilitation context. Native bees can provide reliable local pollination services, and may be a good alternative to managed honey bee or bumble bee colonies for pollination of native plant species.

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Table 3-1. Bee species collected visiting *A. filipes* flowers in wild populations and agricultural settings. Nesting biology info from Clement & Rust (1976), Michener (2000), Cane et al. (2007). *NTN = Cane et al. (2007) stated that this species has never been collected in trapnests despite extensive sampling. Records on other species of *Astragalus*: C = Clement et al. (2006), Ge = Geer et al. (1995), Gr = Green & Bohart (1975), K = Karron (1987), R = Richards (1987), S = Sugden (1985), T = Tepedino & Geer unpublished manuscript. Honey bees were absent from all wild populations of *A. filipes.*

Family	Bee species	# individuals collected or observed	# locations collected or observed	Nesting biology	Previous studies
ANDRENIDAE					
	Andrena sp.	1	1	ground	
	Perdita wyomingensis Cockerell	1	1	ground	
APIDAE					
	Anthophora sp.	1	1	ground	
	Anthophora urbana Cresson	3	3	ground	
	Anthophora ursina Cresson	3	3	ground	C,Gr
	Apis mellifera L.	208	2	hive - managed	C,Ge,Gr,S,T
	Bombus bifarius Cresson	2	2	colony - ground	C,Ge,R
	Bombus californicus Smith	1	1	colony - ground	C,R
	Bombus centralis Cresson	12	7	colony - ground	C,Gr,R
	Bombus fervidus (Fabricius)	4	4	colony - ground	C,Ge,Gr,R
	Bombus huntii Greene	18	8	colony - ground	C,Ge,Gr,K,R,S
	Bombus nevadensis Cresson	13	3	colony - ground	C,Ge,Gr,R,S
	Bombus vosnesenskii Radoszkowski	2	1	colony - ground	S
	Bombus (Psithyrus) insularis (Smith)	1	1	colony - ground	Gr,R
	Eucera sp.	2	2	ground	
	Eucera actuosa (Cresson)	2	2	ground	C
	Eucera edwardsii (Cresson)	6	4	ground	C,Gr
	Eucera frater (Cresson)	27	9	ground	C
	Melecta pacifica Cresson	2	1	ground	T
	Nomada sp.	1	1	ground	
COLLETIDAE					
	Hylaeus sp.	1	1	ground	

Family	Bee species	# individuals collected or observed	# locations collected or observed	Nesting biology	Previous studies
HALICTIDAE					
	Agapostemon angelicus/texanus Cockerell/Cresson	2	2	ground	C
	Dialictus sp.	6	5	ground	
	Halictus rubicundis (Christ)	1	1	ground	C,Gr
	Halictus tripartitus Cockerell	11	4	ground	
	Lasioglossum sisymbrii (Cockerell)	2	1	ground	T
MEGACHILIDAE					
	Anthidium sp.	1	1	unknown	
	Anthidium atripes Cresson	5	3	unknown	
	Anthidium utahense Swenk	3	2	ground, cavity, trapnest	
	Ashmeadiella sculleni Michener	2	1	wood	
	Hoplitis sp.	4	2	unknown	
	Hoplitis hypocrita (Cockerell)	21	9	stem	C
	Megachile sp.	3	1	unknown	
	Osmia sp.	2	2	unknown	
	Osmia sp. A	2	2	unknown	
	Osmia sp. B	1	1	unknown	
	Osmia sp. C	1	1	unknown	
	Osmia aff. hurdi	6	1	unknown	
	Osmia albolateralis Cockerell	9	5	stem	C
	Osmia alpestris Rust & Bohart	1	1	unknown	
	Osmia atrocyanea Cockerell	8	4	wood	C,Gr
	Osmia brevis Cresson	5	4	unknown *NTN	
	Osmia bruneri Cockerell	32	13	wood, stem, trapnest	C
	Osmia calla Cockerell	2	2	unknown	
	Osmia cyanella Cockerell	1	1	unknown	
	Osmia cyanopoda Cockerell	3	2	unknown	C,Ge
	Osmia densa Cresson	1	1	wood	
	Osmia ednae Cockerell	2	2	unknown	
	Osmia granulosa Cockerell	1	1	unknown	
	Osmia hurdi White	5	2	unknown	Ge
	Osmia integra Cresson	4	2	soil, surface?	C,Gr,T

Family	Bee species	# individuals collected or observed	# locations collected or observed	Nesting biology	Previous studies
	Osmia longula Cresson	6	2	surface	Ge
	Osmia n. sp. 1 nr. sladeni	6	2	unknown	C
	Osmia n. sp. 2 nr. sladeni	1	1	unknown	
	Osmia nanula Cockerell	2	2	unknown	
	Osmia nemoris Sandhouse	4	2	trapnest, stem	
	Osmia nifoata Cockerell	9	6	ground	
	Osmia nigrifrons Cresson	15	7	stem	C,Ge,Gr,T
	Osmia "parkeri" Griswold	1	1	unknown	
	Osmia physariae Cockerell	10	4	unknown	
	Osmia pusilla Cresson	1	1	wood, stem	C,Ge
	Osmia raritatis Michener	8	6	unknown	
	Osmia rawlinsi Sandhouse	5	3	unknown	
	Osmia regulina Cockerell	3	1	unknown	C
	Osmia simillima Smith	1	1	gall, wood	C
	Osmia trevoris Cockerell	14	6	unknown *NTN	C
	Osmia unca Michener	1	1	soil, trapnest	C

Table 3-2. Foraging tempo of bees observed visiting *A. filipes*. N is the number of individual bees observed. Time is in seconds per flower, with standard deviation. Times followed by different letters are significantly different (P<0.05) (REGWQ).

Bee species	*N*	*A. filipes* time
Bombus nevadensis queens	11	2.6 ± 0.9^{B}
Osmia sanrafaelae	29	5.9 ± 1.2^{A}
Apis mellifera	28	6.0 ± 1.8^{A}
Hoplitis hypocrita	38	6.4 ± 2.4^{A}
Osmia bruneri	95	7.1 ± 2.7^{A}

Table 3-3. Collection locations of the six most common bee species on *A. filipes*. Locations listed by county with State in bold. Some species were collected at multiple locations within a single county.

Bee species	Locations
Osmia bruneri Cockerell	**ID**: Butte, Owyhee; **NV**: Elko, Lander; **OR**: Harney, Malheur; **UT**: Cache.
Hoplitis hypocrita (Cockerell)	**CA**: Modoc; **ID**: Owyhee; **NV**: Elko; **OR**: Malheur; **UT**: Cache.
Eucera frater (Cresson)	**CA**: Lassen, Modoc; **ID**: Ada; **NV**: Lander; **OR**: Malheur; **UT**: Cache.
Bombus huntii Greene	**CA**: Modoc; **ID**: Owyhee; **NV**: Elko, Lander; **OR**: Malheur; **UT**: Cache.
Osmia nigrifrons Cresson	**CA**: Modoc; **ID**: Owyhee; **NV**: Lander; **OR**: Malheur; **UT**: Cache.
Bombus centralis Cresson	**CA**: Lassen; **ID**: Ada, Owyhee; **NV**: Lander; **OR**: Malheur.

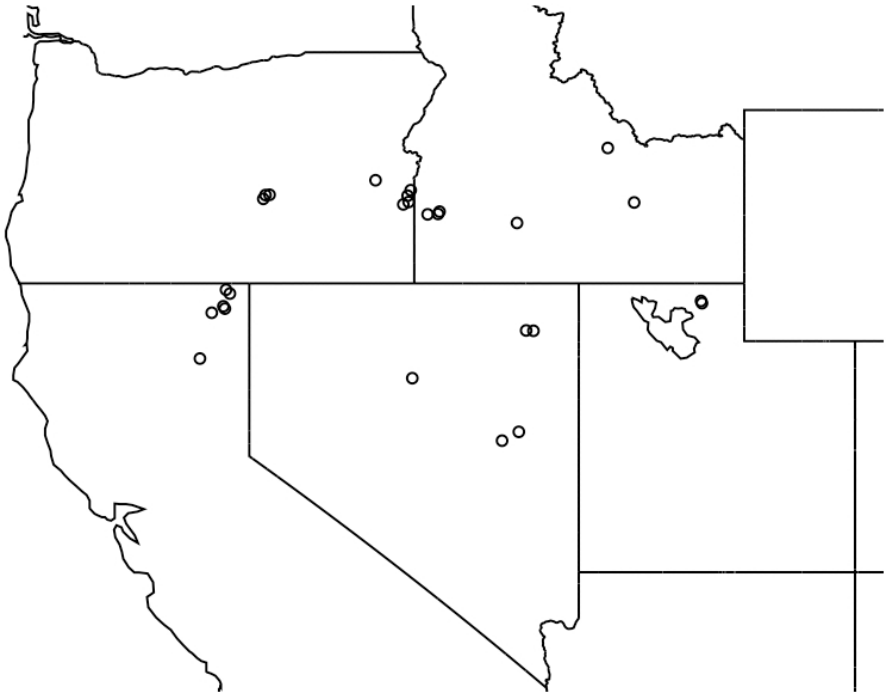

Figure 3-1. Map of bee survey locations on *A. filipes*. Surveys were conducted at wild *A. filipes* populations, except for two cultivated locations in Cache County, Utah.

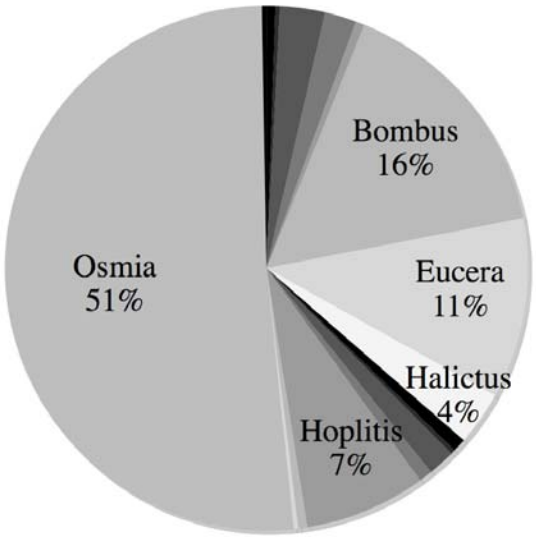

Figure 3-2. Percentages of bee genera collected on *A. filipes*. The five most common genera are listed. Honey bees (*Apis mellifera*) were excluded from this figure.

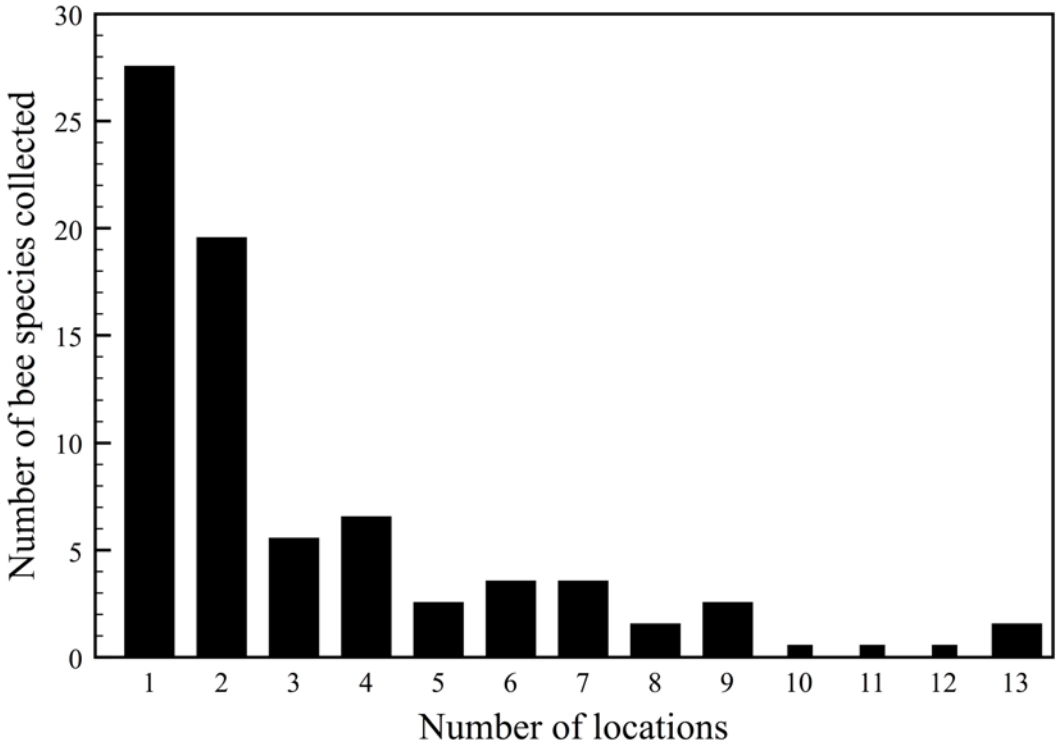

Figure 3-3. Number of bee species collected at different locations. Most species were collected at 1-2 locations. Only one species collected at all 13 locations.

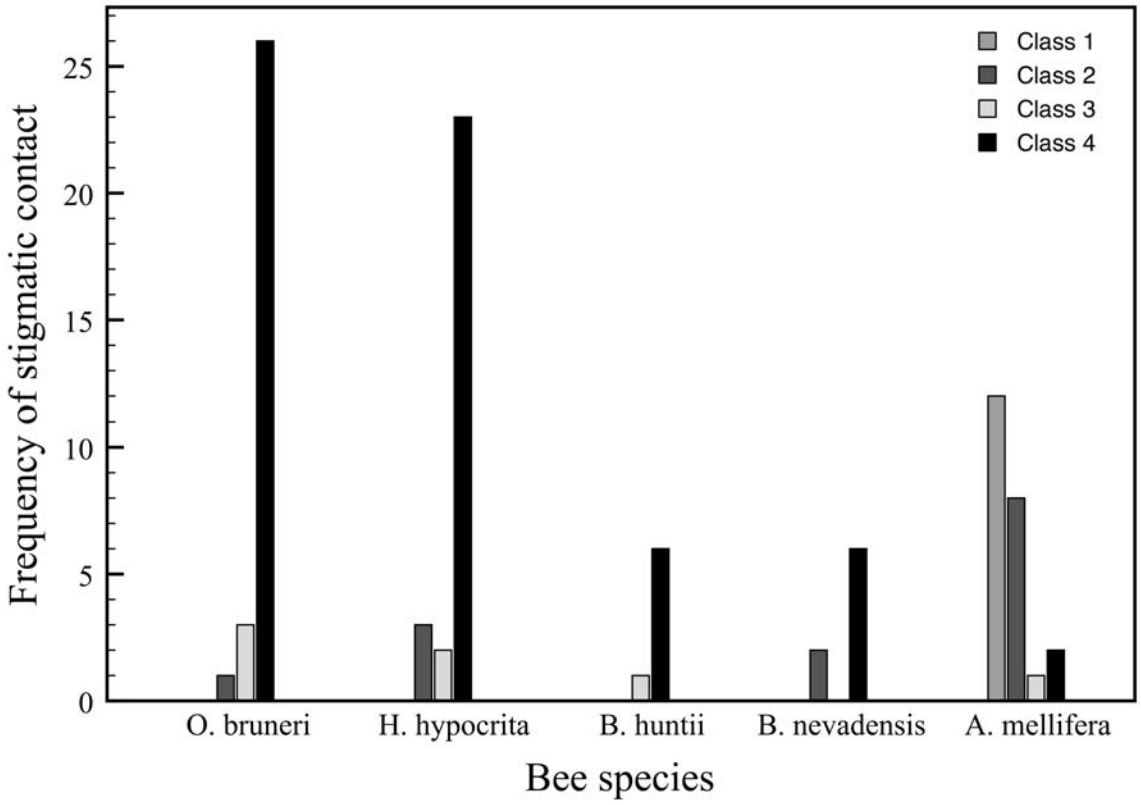

Figure 3-4. Frequency of stigmatic contact for all species compared. Classes are as follows: 1) stigmatic contact was observed in <70% of visits; 2) 70-89% of visits; 3) 90-99% of visits; 4) 100% stigmatic contact observed. Frequency of stigmatic contact was significantly different between species ($P<<0.0005$). Observations of *B. huntii* included queens and workers, while samples for *B. nevadensis* included only queens.

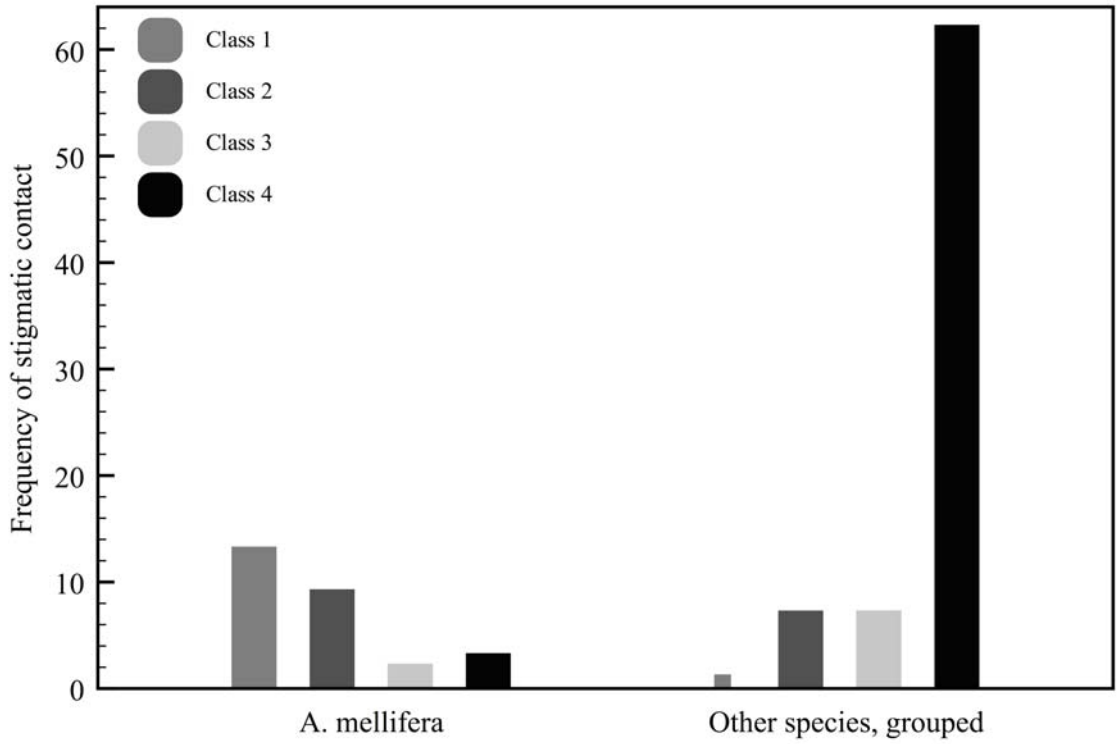

Figure 3-5. Frequency of stigmatic contact for honey bees (*A. mellifera*) versus all other species (*Bombus huntii*, *B. nevadensis* queens, *Hoplitis hypocrita* & *Osmia bruneri*) together. Classes are as follows: 1) stigmatic contact was observed in <70% of visits; 2) 70-89% of visits; 3) 90-99% of visits; 4) 100% stigmatic contact observed. There is a significant difference in frequency of stigmatic contact of honey bees compared to other bees ($P<<0.0005$).

CHAPTER FOUR

CONCLUSIONS

Farming of *Astragalus filipes* for seed production will require attention to bee pollination to maximize seed set. We found that *A. filipes* has a mixed mating system wherein self-pollination results in limited seed set, while outcrossing greatly enhances seed production. Very little seed results without pollinator visitation. Pollen transfer between plants representing geographically distant seed accessions (xenogamy) resulted in an unexpected slight decrease in seed germination, but no difference in fruit or seed set. Overall, xenogamous pollen transfer is necessary to maximize seed production. It is thus important to include pollinators in management considerations for *A. filipes*.

A diversity of bees visit flowers of *A. filipes* in wild and cultivated locations. Bees in the genus *Osmia* were the dominant group of floral visitors in both abundance and diversity, comprising over half of the species collected. Common floral visitors were assessed for their floral handling behaviors on *A. filipes*. Most species did not differ in floral handling behaviors, and seemed proficient enough to be considered as legitimate pollinators. Bumble bee queens (*Bombus nevadensis*) were significantly faster floral visitors than the other species observed; however, bumble bee queens are only active floral visitors for a small portion of the year before they retire to their nests full-time. Honey bees (*Apis mellifera*) seemed to be the poorest pollinators of those that we surveyed, as they more frequently failed to contact the flowers' sexual column than any other bee species assessed.

Bees collected on *A. filipes* vary considerably in their nesting biologies. The wide array of nesting biologies present in this pollinator guild may help to make it more resilient to perturbations. It is also more likely that at least a subset of its pollinators will survive occasional rangeland fires. Knowledge of the dominant pollinators' nesting biologies helps us to target more easily managed species for active management on farms.

This study will help researchers and growers developing *A. filipes* for seed production to more effectively manage the plants and their pollinators for maximal seed set. Our research will also be useful to managers seeding out *A. filipes* in the Great Basin at rehabilitation sites, where the surviving pollinator guilds of this locoweed will be needed for sustained reproduction in these rehabilitation plantings.